Solid Phase Extraction of Lysergides from Biological Samples

1 Introduction

Lysergic acid diethylamide (LSD) is one of the most potent hallucinogenic drugs. Detection of LSD in biological samples can be an analytical challenge due to the low doses consumed and extensive metabolism. LSD's major metabolite 2-oxo-3-hydroxy LSD (OH-LSD) is detectable in urine at 16-43 times the concentration of LSD; to date OH-LSD has never been detected in blood.

2 Scope

This procedure qualitatively detects LSD in blood and urine. It also qualitatively detects its primary metabolite, OH-LSD, in both blood and urine. It is derived from "LC-MS Analysis of 2-oxo-3-hydroxy LSD from Urine Using a Speedisk® Positive-Pressure Processor with Cerex® PolyChrom™ CLIN II Columns" and "Liquid Chromatography-Electrospray Ionization Mass Spectrometry for the Detection of Lysergide and a Major Metabolite, 2-Oxo-3-Hydroxy-LSD, in Urine and Blood". The solid phase extraction (SPE) scheme is followed essentially intact. The analysis of LSD and its major metabolite OH-LSD are performed by liquid chromatography-electrospray ionization-high resolution mass spectrometry.

3 Principle

Blood and urine specimens can be selectively assayed for OH-LSD and LSD by this method. Following the addition of internal standard (d_3-LSD), blood and/or urine samples are buffered and extracted with Cerex® PolyChrom™ CLIN II solid phase columns. LSD and OH-LSD are collected in the same fraction and taken to dryness. Once dry, the eluent is reconstituted in 20% acetonitrile. Samples are analyzed by liquid chromatography-electrospray ionization-high resolution mass spectrometry (LC-ESI-FT-MS/MS) for both LSD and OH-LSD.

4 Specimens

This procedure uses 2 mL of blood or urine.

5 Equipment/Materials/Reagents

a. 16x100 mm screw-top tubes with caps

b. 12x75 mm and 16x100 mm culture tubes with snap-tops

c. Routine laboratory supplies and equipment, including disposable pipets, graduated cylinders, volumetric flasks, pH paper, vortex mixer, centrifuge, etc.

d. Positive pressure solid-phase extraction manifold with nitrogen supply

e. Cerex® PolyChrom™ CLIN II solid phase columns (50mg) 6mL

f. Evaporator with nitrogen supply

g. Liquid chromatography Fourier transform mass spectrometry system (LTQ Orbitrap-XL®, or equivalent)

h. HPLC column (Grace Alltima® C18, 150x2.1 mm, 5 µm d_p, or equivalent with pre-filter)

i. Acetonitrile (Optima grade)

j. Methanol (HPLC and Optima grades)

k. Water (deionized and Optima grade)

l. Ethyl Acetate (HPLC grade)

m. Concentrated Hydrochloric Acid (HCl) (certified ACS grade)

n. Concentrated Ammonium Hydroxide (certified ACS grade)

o. Concentrated Formic Acid (Puriss grade)

p. Concentrated Acetic Acid (certified ACS grade)

q. Ammonium Formate (99.99+ %)

r. Sodium Phosphate, Monobasic, Monohydrate (certified ACS grade)

s. Sodium Phosphate, Dibasic, Heptahydrate (certified ACS grade)

t. Anhydrous Potassium Carbonate (certified ACS grade)

u. Anhydrous Potassium Bicarbonate (certified ACS grade)

v. Phosphate Buffer (0.1 M, pH 6):
To a 1-L volumetric flask, add 900 mL DI (deionized) water. Add 12.2 g sodium phosphate, monobasic, monohydrate and 3.2 g sodium phosphate, dibasic, heptahydrate and mix well to dissolve. Verify pH ~6 and dilute to the mark with DI water. Store refrigerated in glass. Stable for 2 months.

w. Carbonate Buffer (0.27 M, pH 9):
To a 250-mL volumetric flask, add 200 mL DI water. Add 2.5 g anhydrous potassium carbonate and 5 g anhydrous potassium bicarbonate and mix well to dissolve. Adjust pH to ~9 with concentrated acetic acid or concentrated ammonium hydroxide and dilute to the mark with DI water. Store refrigerated in glass. Stable for 1 month.

x. 0.1 M Hydrochloric Acid:
In a 100-mL graduated cylinder, dilute 0.8 mL of concentrated hydrochloric acid to 96 mL with DI water and mix well. Store in glass at room temperature. Stable for 6 months.

y. Elution Solvent (25:1 ethyl acetate:ammonium hydroxide):
Measure 75 mL of ethyl acetate into a 100-mL graduated cylinder. Add 3 mL of concentrated ammonium hydroxide and mix well. Prepare fresh daily.

z. Reconstitution Solvent (20:80 acetonitrile:water):
Combine 4 mL acetonitrile with 16 mL water (Optima grade) and mix well. Store in glass at room temperature. Stable for 6 months.

aa. LC Mobile Phase #1 (20 mM ammonium formate, pH 4.4):
Add 1.26 g ammonium formate to 1 L of water (Optima grade). Mix well to dissolve, and filter. Add 70 µL concentrated formic acid and verify pH ~4.4, adjusting with concentrated formic acid or concentrated ammonium hydroxide as necessary. Store in glass at room temperature. Stable for 1 month.

6 Standards and Controls

a. d_3-LSD Stock Standard (100 µg/mL):
Purchased from Cerilliant or another approved vendor. Stability and storage determined by manufacturer.

b. d_3-LSD Working Internal Standard (100 ng/mL):
To a 25-mL volumetric flask, add 25 µL of the d_3-LSD Stock Standard. Dilute to the mark with acetonitrile and mix well. Store below 0ºC in glass. Stable for at least 1 year.

c. LSD Stock Standard (1 mg/ml):
Purchased from Cerilliant or another approved vendor. Stability and storage determined by manufacturer.

d. LSD Intermediate Working Solution (2 µg/ml):
To a 10-mL volumetric flask, add 20 µL of the LSD Stock Standard. Dilute to the mark with acetonitrile and mix well. Store below 0ºC in glass. Stable for at least 1 year.

e. OH-LSD Stock Standard (100 µg/ml):
Purchased from Cerilliant or another approved vendor. Stability and storage determined by manufacturer.

f. OH-LSD Intermediate Working Solution (2 µg/ml):
To a 10-mL volumetric flask, add 200 µL of the OH-LSD Stock Standard. Dilute to the mark with acetonitrile and mix well. Store below 0ºC in glass. Stable for at least 1 year.

g. Lysergide Control Working Solution (20 ng/ml LSD; 40 ng/ml OH-LSD):
To a 25-mL volumetric flask, add 250 µL of the LSD intermediate Working Solution and 500 µL of the OH-LSD Intermediate Working Solution. Dilute to the mark with acetonitrile and mix well. Store below 0ºC in glass. Stable for at least 1 year.

h. Lysergic Acid Methypropylamide (LAMPA) Stock Standard (1 mg/ml):
Purchased from Cerilliant or another approved vendor. Stability and storage determined by manufacturer.

i. LAMPA Intermediate Working Solution (2 µg/ml):
To a 10-mL volumetric flask, add 20 µL of the LAMPA Stock Standard. Dilute to the mark with acetonitrile and mix well. Store below 0ºC in glass. Stable for at least 1 year.

j. iso-LSD Stock Standard (100 µg/ml):
Purchased from Cerilliant or another approved vendor. Stability and storage determined by manufacturer.

k. iso-LSD Intermediate Working Solution (2 µg/ml):
To a 10-mL volumetric flask, add 200 µL of the iso-LSD Stock Standard. Dilute to the mark with acetonitrile and mix well. Store below 0ºC in glass. Stable for at least 1 year.

l. Lysergide Testmix Stock Solution (25 ng/ml each component):
To a 10-mL volumetric flask, add 125 µL each of the OH-LSD, LAMPA, and iso-LSD Intermediate Working Solutions. Dilute to the mark with acetonitrile and mix Well. Store below 0ºC in glass. Stable for at least 1 year.

m. LC-MS Performance Standard (5 ng/ml per component):
Combine 30 µL of the Lysergide Testmix Stock Solution with 120 µL Optima-grade water and mix well. Prepare fresh daily. A 10 µL portion of this standard is analyzed before each day's samples, in order to confirm acceptable instrument performance.

n. Negative Control Blood and/or Urine:
Purchased from Cliniqa, UTAK, Dynatek, or another approved vendor or prepared in-house from an appropriate blank specimen. Stability and storage determined by manufacturer. A negative control will be analyzed with every assay. When possible, the negative control will be matrix matched.

o. Positive Control Blood and/or Urine (0.5 ng/ml LSD; 1 ng/ml OH-LSD):
These are normally prepared in-house, but may be purchased from an approved vendor as needed. Normally prepared the day of extraction by adding 50 µL of Lysergide Control Working Solution to 2 mL of Negative Control Blood or Urine.

7 Sampling

Not applicable.

8 Procedure

Appendix 1 contains an abbreviated version of this procedure. This form may be used at the bench by the examiner or chemist performing the procedure.

a. To properly labeled 16 x 100 mm screw-cap tubes add 2 mL of specimen or control.

b. Add 20 µL of Internal Standard Working Solution and vortex briefly.

c. Add 2 mL of 0.1 M, pH 6 Phosphate Buffer and vortex for 30 seconds.

d. For blood and turbid urine specimens:

Centrifuge for 15 min at 3500 rpm. Decant supernatant into a 16 x 100 mm culture tube.

For non-turbid urine specimens, skip to step e.

e. Place Cerex® PolyChrom™ CLIN II solid phase columns (50 mg) 6 mL onto SPE positive pressure manifold.

f. Load sample on SPE cartridges.

g. Push sample through column using low range setting (setting 0-2).

h. Wash column with 1 mL Potassium Carbonate Buffer ($K_2CO_3/KHCO_3$) (pH 9) (setting 2-3).

i. Wash column with 2 mL 0.1M HCl (setting 2-3).

j. Wash column with 1 mL Methanol (setting 2-3).

k. Wash with 3 mL Ethyl Acetate (setting 2-3).

l. Elute with 4 mL Elution Solvent into 12 x 75 mm test tubes by gravity.

m. Evaporate to dryness under nitrogen at 55ºC.

n. Reconstitute the residue in 100 µL Reconstitution Solvent.

o. Analyze 10 µL of the extract by LC-ESI-FT-MS/MS using the instrumental conditions given in section 9, after verifying that the instrument is in proper working order.

9 Instrumental Conditions

Appendix 2 contains a checklist of method parameters to be used to verify proper instrument conditions prior to the analysis of case samples.

9.1 Liquid Chromatograph Parameters

Column Parameters							
Phase	Alltima® C18	Length	150 mm	Diameter	2.1 mm		
Particle Size	5 µm	Temperature	35°C	Flow Rate	0.3 mL/min		
Mobile Phase A:	Methanol (Optima)	Mobile Phase B:	LC Mobile Phase #1				
Gradient Program – All Ramps Are Linear							
Time (min)	0	3	10	19	20	25	26	31
%A	20	20	35	48	90	90	20	20
%B	80	80	65	52	10	10	80	80

9.2 Mass Spectrometer Parameters

Number of Segments: 4	Ionization Mode: Electrospray Positive Ion	
Segment #1 (0 - 6 min); 1 event		
Full Scan MS	Range: 200-400 m/z	FTMS 15000 resolution
Segment #2 (6 – 13 min); 2 events		
#1: Full Scan MS	Range: 200-400 m/z	FTMS 15000 resolution
#2: Full Scan MS/MS	Precursor: m/z 356.2	Range: 100 – 380 m/z
FTMS 7500 resolution	Isolation Width: 2.0 Da	Collison: CID @ 20
Segment #3 (13 – 20 min); 2 events		
#1: Full Scan MS	Range: 200-400 m/z	FTMS 15000 resolution
#2: Full Scan MS/MS	Precursor: m/z 324.2	Range: 85 – 350 m/z
FTMS 7500 resolution	Isolation Width: 2.0 Da	Collison: CID @ 40
Segment #4 (20 - 31 min); 1 event		
Full Scan MS	Range: 200-400 m/z	FTMS 15000 resolution

10 Decision Criteria

10.1 LC-MS Performance Standard Decision Criteria

10.1.1 Chromatography

In order for the LC to be considered in good operating condition, full MS molecular ion traces for each analyte in the performance standard should generate reasonably symmetric shaped chromatographic peaks. m/z 356.196 is traced for OH-LSD and m/z 324.206 for iso-LSD and LAMPA. The retention times of the 3 analytes should be within ± 5 % of the previous run of the performance standard. LAMPA and iso-LSD should be resolved with at least a 50% drop to baseline when displayed in an extracted ion chromatogram (EIC) with a ±0.005 Da tolerance.

10.1.2 Mass Spectrometry

In order for the MS to be considered in good operating condition, the correct mass assignments for each of the three analytes in the performance standard should be present. The following ions should be present for each analyte. Each observed mass should be within ±0.005 Da of the theoretical value.

OH-LSD:	MS – 356.196	MS/MS – 338.185 (base peak), 265.096, 237.102
Iso-LSD:	MS – 324.206	MS/MS – 281.164 (base peak), 251.117, 223.122, 208.075
LAMPA:	MS – 324.206	MS/MS – 281.164, 251.117, 223.122 (base peak), 208.075

10.2 Batch Acceptance Criteria

d_3-LSD (m/z 327.226) must be detectable in an EIC for every extracted sample. None of the targeted lysergides (LSD, OH-LSD, LAMPA, iso-LSD) may be detected in the Negative Control Sample(s). Both LSD and OH-LSD must be detectable, based upon criteria given in 10.1.2 and using the mass spectral parameters of LAMPA for LSD, in the Positive Control Sample(s).

10.3 Analyte Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. In most cases, all of the below should be met in order to identify LSD or OH-LSD within a biological specimen:

10.3.1 Chromatography

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. In order to be determined acceptable, a chromatographic peak in an unknown sample should compare favorably to a chromatographic peak of the same analyte in a known sample analyzed on the same system in the same or subsequent analytical runs. Additionally, the following two criteria should be met.

10.3.1.1 Retention Time

The retention time of the peak should be within ±5% of the retention time (relative or absolute, as appropriate) obtained from injection of a reference standard or positive control.

10.3.1.2 Signal-to-Noise

To justify the existence of a peak, its baseline signal to peak-to-peak noise ratio should exceed 3. Further, the baseline signal for the peak from the sample of interest should be at least 10 fold greater than that for any observed peak at a similar retention time in a Negative Control or blank sample injected just prior to that sample.

10.3.2 Mass Spectrometry

The MS/MS fragmentation spectra should meet the criteria given in Toxicology SOP 104, *Guidelines for Comparison of Mass Spectra*, with comparison to a contemporaneously analyzed reference standard or positive control.

a. LSD: (fragments of m/z 324.206) The base peak should be m/z 223.122. m/z 281.164 and either 251.117 or 208.075 will be used for the calculation of ion ratios.

b. OH-LSD: (fragments of m/z 356.196) The base peak should be m/z 338.185. m/z 265.096 and 237.102 will be used for the calculation of ion ratios.

11 Calculations

Not applicable.

12 Measurement Uncertainty

Not applicable.

13 Limitations

a. Limit of Detection: LSD: at least 150 pg/mL in blood and urine
OH-LSD: at least 300 pg/mL in blood and urine

b. Interferences: Grossly decomposed or putrefied samples may adversely affect limits of detection. Iso-LSD is an isomer of LSD, and is not chromatographically resolved from

LSD. The two compounds yield the same MS/MS fragment ions, but with radically different ion ratios. The presence of significant levels of iso-LSD in a sample will adversely affect the limit of detection for LSD.

c. LSD is known to be subject to photodegradation. Standards and processed samples should be stored in darkness whenever possible.

14 Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

15 References

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Horn CK, Klette, KL, Stout PR, LC-MS Analysis of 2-oxo-3-hydroxy-LSD from Urine using a Speedisk® Positive Pressure Processor with Cerex® Polychrom™ Clin II Columns," *J Anal Tox* 27: 459-463, (2003).

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Moffat, A.C., *Isolation and Identification of Drugs*, 2nd ed., Pharmaceutical Press: London, 1986.

Winek, C. *Drug and Chemical Blood-Level Data*, 1994.

Performance Monitoring Protocl (QA/QC) for the Thermo LTQ Orbitrap XL LC/MS (ESI) (Inst 207): FBI Laboratory Chemistry Unit – Instrument Operations and Support Subunit Standard Operating Procedures Manual.

Guidelines for Comparison of Mass Spectra (Tox 104): FBI Laboratory Chemistry Unit – Toxicology Subunit Standard Operating Procedures Manual.

FBI Laboratory Safety Manual.

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